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The avian heterophil

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ABSTRACT

Heterophils play an indispensable role in the immune defense of the avian host. To accomplish this defense, heterophils use sophisticated mechanisms to both detect and destroy pathogenic microbes. Detection of pathogens through the toll-like receptors (TLR), FC and complement receptors, and other pathogen recognition receptors has been recently described for the avian heterophil. Upon detection of pathogens, the avian heterophil, through a network of intracellular signaling pathways and the release and response to cytokines and chemokines, responds using a repertoire of microbial killing mechanisms including production of an oxidative burst, cellular degranulation, and production of extracellular matrices of DNA and histones (HETs). In this review, the authors describe the recent advances in our understanding of the avian heterophil, its functions, receptors and signaling, identified antimicrobial products, cytokine and chemokine production, and some of the effects of genetic selection on heterophils and their functional characteristics.

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1. Introduction

Avian species, specifically domestic poultry, are constantly exposed to a myriad of pathogens and microbes in their environments. As seen in mammals, avian species are well armed against foreign microbial invaders, having evolutionarily developed a two-armed approach for dealing with pathogens, manifested in the acquired and innate immune systems. Although often mentioned as two separate pathways, the innate and acquired systems are strategically linked to best protect the host from pathogens and to coexist with normal microbial flora. One of the most important links in the chain of the avian host's immune response to pathogens and an integral part of the avian innate defenses is the heterophil. This granulocytic white blood cell, a counterpart to the mammalian neutrophil, is the first line of defense against invasive pathogens. It is through the heterophil's recognition of and response to pathogens that an effective immune response develops and proceeds towards pathogen elimination.

Previous reviews by Brockus et al. (1998), Harmon (1998) and Maxwell and Robertson (1998) compiled the latest information describing the role of heterophils in inflammation and disease and their basic functions and characteristics. In the 14 years since these reviews, a considerable amount of research has been conducted and has added to our understanding of the avian heterophil and its characteristics and functions. In this review, we will discuss the more recently described attributes of the avian heterophil.

including its functions, receptors, intracellular signaling, antimicrobial products, and the known genetics related to its functional efficiency.

2. Heterophil functions and antimicrobial products

2.1. Phagocytosis

Phagocytosis is an active, receptor-mediated internalization of pathogens by phagocytes such as neutrophils in mammalian and heterophils in avian species. Fcγ (antibody) and complement (complement cascade) receptors expressed on the phagocytes mediate opsonization-dependent phagocytosis, while receptors such as dectin-1, scavenger receptors, and mannose receptors mediate nonopsonic phagocytosis (Flannagan et al., 2012). Once phagocytized, pathogens are entrapped within a membrane bound vacuole called the phagosome. The event quickly triggers the onset of fusion of cytoplasmic granules with the phagosome and results in killing of entrapped pathogens by microbicidal substances released or produced by the processes of degranulation and the oxidative burst. Therefore, phagocytosis is, generally, immediately followed by degranulation and production of an oxidative burst which often occur simultaneously. Avian heterophils are the predominate granulocytes in circulating blood of most birds and they are highly phagocytic (Maxwell and Robertson, 1998). In poultry, these granulocytic phagocytes are rapidly recruited to the site of infection where they engage in phagocytosis and killing of pathogens (Kogut et al., 1994, 1995a,b, 1998a,b; Stabler et al., 1994). Chicken heterophils are capable of phagocytizing effectively both opsonized and nonopsonized pathogens, such as *Salmonella*;

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however, the resulting immune response as measured by cytokine mRNA expressions is different. *Salmonella* opsonized with normal chicken serum (complement receptor) exhibited decreased mRNA expression of IL-1 β and IFN- γ and increased mRNA expression of TGF- β 4 compared to *Salmonella* opsonized with IgG (Fc γ R) while there were no differences in mRNA expression of IL-6, IL-8(also named Cxcli2), and IL-18 (Kogut et al., 2003a). Priming of heterophils with recombinant chicken IL-2 did induce increased expression of IL-8 and IL-18 regardless of opsonization with complement of antibody (Kogut et al., 2003b). These results suggest that different signaling pathways are associated with opsonic and nonopsonic phagocytosis (Kogut et al., 2003a,b).

2.2. Degranulation

Degranulation is the process by which heterophils release granule substances into the external environment at the site of the infection in response to microbial agonist stimulation or into the pathogen containing phagosome, which plays an important role in host defense and inflammation. Granules contain substances including antimicrobial proteins, peptides, adhesion molecules, enzymes, receptors, and other toxic mediators. Heterophils utilize the cytoplasmic granules to allow a controlled release of potentially toxic substances at the site of infection to fight pathogens while mitigating damage to host tissue. In mammalian neutrophils, granules are highly heterogeneous with regard to their structure, content, and function and are subdivided into four distinct granules which are formed sequentially during neutrophil maturation: myeloperoxidase-positive granules also called primary or azurophil granules, myeloperoxidase-negative granules termed specific or secondary granules, tertiary or gelatinase granules with a high content of gelatinase, and lastly secretory vesicles (Borregaard et al., 2007). Upon stimulation, secretory vesicles and tertiary granules are rapidly released, followed by secondary granules and primary granules last. The granule contents and functions of mammalian neutrophils are well defined: secretory and tertiary vesicles contain proteins and receptors needed for neutrophil adhesion and migration; secondary granules contain antimicrobial proteases and peptides that can kill pathogens and matrix metalloproteases that aid migration; and primary granules contain proteolytic enzymes and antimicrobial peptides that are secreted into the phagocytic vacuole to kill microorganisms Borregaard et al., 2007).

However, granules in avian heterophils are not as clearly defined. Three types of granules have been described in avian heterophils: large, rod-shaped, dense (Type I); medium sized, oval, light (Type II); and small-core (Type III) (Daimon and Caxton-Martins, 1977). The granule contents of heterophils are mostly unknown; excepting for these reported in early studies, including β -defensins (Gal-1 and Gal-2), cathepsin, lysozyme, acid phosphatase, β -glucuronidase, and α -glucosidase (Daimon and Caxton-Martins, 1977; Evans et al., 1994, 1995; Harwig et al., 1994; Macrae and Spitznagel, 1975; Brockus et al., 1998; Brune and Spitznagel, 1973; Fujimori et al., 1978; Osculati, 1970; Maxwell, 1984). In a recent study, elastase, an enzyme found in primary granules in neutrophils, was identified to be a constituent of the fibrous structures, heterophil extracellular traps (HET), released by chicken heterophils upon stimulation (Chuammitri et al., 2009). The most notable contrast to neutrophils is the lack of the enzyme, myeloperoxidase in heterophils granules. Additionally, alkaline phosphatase and catalase are also absent from the granules of avian heterophils (Daimon and Caxton-Martins, 1977). Although there are at least 14 chicken β -defensins (van Dijk et al., 2008), only Gal-1 and Gal-2 were positively identified and associated with heterophil granules (Evans et al., 1994; Harwig et al., 1994; Macrae and Spitznagel, 1975; Brockus et al., 1998). A recent study showed that

chicken cathelicidin-2, a member of another family of antimicrobial cationic peptides, is abundantly present in the Type I granules of chicken heterophils; and that the mature cathelicidin-2 is released upon stimulation of heterophils with *Salmonella* lipopolysaccharide (LPS) (van Dijk et al., 2009). In avian heterophils, degranulation is closely associated with phagocytosis which is evident that both opsonized and nonopsonized bacteria stimulate significant degranulation activity as measured by β -glucuronidase activity in the reaction medium (Kogut et al., 2001a,b; Kogut et al., 2003a,b). Various constituent microbial molecules, such as LPS, lipoteichoic acid (LTA), peptidoglycan (PGN), and flagellin (FGN) are also found to stimulate degranulation in chicken heterophils (Kogut et al., 2005a,b). These microbial agonists were found to be ineffective stimulators of degranulation in heterophils from commercial turkeys, although there was significant degranulation activity when challenged with whole bacteria (He et al., 2008).

Although not specifically defined as a degranulation event, the production of HETs is most likely associated with degranulation, as seen with mammalian and fish neutrophil NETs (neutrophil extracellular traps) (Chuammitri et al., 2009; Brinkmann et al., 2004; Fuchs et al., 2007; Gupta et al., 2006, 2005; Lippolis et al., 2006; Palic et al., 2007). This process is also linked to the production of an oxidative burst in mammals (Ermert et al., 2009). HETs have been characterized and shown to contain DNA and histones and are believed to act similarly to NETs, trapping and killing invading microbes and, subsequently the neutrophil itself (Chuammitri et al., 2009; Brinkmann et al., 2004; Fuchs et al., 2007; Lippolis et al., 2006; Ermert et al., 2009; Brinkmann and Zychlinsky, 2007).

2.3. Oxidative burst

Phagocytic leukocytes undergo oxygen-dependent production of reactive oxygen species (ROS) in response to external stimuli, such as microbes and microbial associated molecules, in a process referred to as an oxidative or respiratory burst. The oxidative burst is catalyzed by a multi-protein complex NADPH oxidase which exists in the inactive, dissociated state in resting cells. Upon stimulation, cytosolic subunits p47/p67/p40 complex are assembled with the gp91/p22 complex residing on membranes of granules into an active NADPH oxidase which then generates superoxide anion (O_2^-) (Winterbourn and Kettle, 2012). The superoxide anion (O_2^-) is then converted to hydrogen peroxide (H_2O_2) with either spontaneous dismutation itself or with facilitation of superoxide dismutase (SOD). In mammals, Both O_2^- and H_2O_2 can be further converted by myeloperoxidase (MPO) to strong antimicrobial and toxic hypochlorous acid (HOCl), hydroxyl radical (OH^-), singlet oxygen (1O_2), and ozone (O_3) (Klebanoff, 2005).

Due to a lack of MPO, avian heterophils generate a relatively weak oxidative response compared to the activity of mammalian neutrophils and fail to produce increased amounts of hydrogen peroxide or peroxide anion (Wells et al., 1998). However, despite the lack of an efficient oxidative antimicrobial mechanism, heterophils are quite effective in phagocytizing and killing bacteria (Wells et al., 1998; Genovese et al., 1999). It appears that chicken heterophils depend primarily on nonoxidative microbicidal mechanism to kill microorganisms by utilizing antimicrobial proteins [etc. lysozyme (Daimon and Caxton-Martins, 1977) and elastase (Chuammitri et al., 2009)], peptides [etc. β -defensins (Evans et al., 1994; Harwig et al., 1994; Macrae and Spitznagel, 1975; Brockus et al., 1998)] and cathelicidins (van Dijk et al., 2009), and possibly HET (Chuammitri et al., 2009). Unlike neutrophils, avian heterophils do not respond to stimulation with formyl-methionyl-leucyl-phenylalanine (fMLP), a chemotactic peptide, in regard to degranulation and oxidative burst, suggesting the lack of the fMLP receptor in avian heterophils (Kogut et al., 1998a;

Gerilechaogetu et al., 2009). It is well known that NADPH oxidase is activated by protein kinase C (PKC) (Nixon and McPhail, 1999). Phorbol myristate acetate (PMA), a PKC activator, is a most powerful stimulant for oxidative burst in both neutrophils (Nixon and McPhail, 1999) and avian heterophils (He et al., 2008; Kogut et al., 1998a,b), indicating the mechanism involved in activation of NADPH oxidase is conserved between chicken heterophils and mammalian neutrophils. Avian heterophils' oxidative burst in general is responsive to stimulation with bacteria and microbial agonists of the innate immune receptors, such as toll-like receptors (TLRs) (Kogut et al., 2005a,b, 1998a,b; He et al., 2003, 2008; Farnell et al., 2003).

2.4. Heterophil functions early In life

Neonatal life, whether in mammalian or avian species, is defined, immunologically, by a naïve and inefficient immune response. Newly hatched chicks and turkeys have been shown to have decreased innate immune functions during the first days and weeks after hatch. Specifically, heterophils from young poultry are functionally deficient, as demonstrated by reduced microbial killing, phagocytosis of bacteria, cellular degranulation, and oxidative burst production (Genovese et al., 2000, 1998; Wells et al., 1998; Lowry et al., 1997). Heterophil functions in 1–14 day-old commercial turkeys and layer chickens were found to be inefficient compared to older birds (Genovese et al., 2000, 1998; Wells et al., 1998; Lowry et al., 1997). The inefficiency in function continued until approximately 21 days-of-age and was correlated with an increase in susceptibility to infection with pathogens such as *Salmonella* (Lowry et al., 1997; Genovese et al., 2000, 1998). The reduced levels of heterophil functions observed in commercial turkey and chicken lines may suggest an inherent susceptibility to bacterial infection in young poultry and these deficiencies may be a contributing factor to health problems later in life.

3. Genetic selection effects

3.1. Heterophil functions

Commercial lines of poultry have been selected for certain production traits over time: laying breeds for egg production, broiler and turkey breeds for meat production. The effects of commercial selection on the innate immune response, heterophils specifically, in selected breeds have recently been investigated including characterization of the heterophil-mediated innate immune response of parental broilers (lines A and B) and their F1 reciprocal crosses (C = B sire × A dam; D = A sire × B dam). Heterophils isolated from line A (fast-feathering chickens) are functionally more effective at phagocytizing and killing bacteria that is associated with an increased ability to degranulate and production of a greater oxidative burst response compared to heterophils from line B (slow-feathering chickens) (Swaggerty et al., 2003b). The gene associated with fast and slow feathering in avian species (located on the Z sex chromosome) has been associated with immune functions, and specifically heterophil functions (Smith and Crittenden, 1998; Swaggerty et al., 2003b). Examination of a second pair of fast and slow-feathering lines (X and Y, respectively) demonstrated the same trend of improved functional efficiency in heterophils from the fast-feathering line of birds compared to the slow-feathering line (Swaggerty et al., 2003a). Results further showed both biochemical killing mechanisms of degranulation and oxidative burst were linked to the feathering gene while phagocytosis and cytokine/chemokine mRNA expression were not (Swaggerty et al., 2006a).

In addition to differences between broiler lines, there are numerous studies comparing different breeds of chickens and tur-

keys. A study by Redmond and colleagues showed the heterophil functional phenotypes of phagocytosis and bacterial killing vary depending on the breed/cross of chickens (Redmond et al., 2011a). Comparison of heterophils between three distinct breeds of chickens that included Leghorns, broilers, and the Fayoumi line shows that selection for egg production, meat production, and no selection pressures, respectively, has produced birds whose heterophils are significantly different with respect to phagocytosis and HET production which is associated with catching and killing invading microorganisms (Chuammitri et al., 2011). Comparisons in turkeys also demonstrated differences between heterophil functional efficiency. Heterophils isolated from small-bodied wild-type turkeys were found to be functionally more efficient with respect to degranulation and oxidative burst compared to those isolated from large commercial heavy-bodied turkeys (Genovese et al., 2006) suggesting selection pressures for growth have adversely affected immune competence.

3.2. Gene expression

Cytokines and chemokines are essential effector molecules produced by cells during innate and acquired immune responses and are involved in initiating and coordinating immune responses aimed at eradicating pathogens. Pro-inflammatory cytokines and chemokines have key roles in initiating an innate immune response and assist in generating local inflammatory responses. In addition to basic functions, heterophils also express key cytokines and chemokines are influential in determining overall resistance and/or susceptibility to foodborne and poultry pathogens. Further evaluation of the parental line of broilers (A and B) and F1 crosses (C and D) showed a distinct heterophil-mediated cytokine/chemokine profile is observed between the lines and crosses at basal levels and following *in vitro* stimulation and *in vivo* challenge with *Salmonella enteritidis*. Ferro and colleagues demonstrated that differences in resistance to *S. enteritidis* were accompanied by increases in heterophil mRNA expression of pro-inflammatory mediators including interleukin (IL)-6, IL-1 β , and IL-8 (Ferro et al., 2004). Evaluation of baseline levels of various cytokines and chemokines in heterophils from non-challenged chickens showed the differences occur as early as one day post-hatch where heterophils from line A had higher levels of IL-6 and IL-8 compared to those from line B (Swaggerty et al., 2004). Isolated heterophils were also subjected to exposure to *S. enteritidis* and the differences were expanded to include a significant up-regulation of IL-18 while transforming growth factor (TGF)- β 4 levels were significantly higher in heterophils isolated from line B and cross C chickens compared to A and D, respectively (Swaggerty et al., 2004) and this trend was maintained out to one month of age (Swaggerty et al., 2006b). The differential cytokine/chemokine profile between heterophils from lines A and B was also reported following stimulation with various TLR agonists (Kogut et al., 2006).

There are numerous *in vitro* studies evaluating cytokine/chemokine mRNA expression by heterophils isolated from different breeds of chickens following stimulation with *S. enteritidis*. Comparison of the mRNA expression of IL-10, IL-6, TGF- β 4, and granulocyte macrophage-colony stimulating factor (GM-CSF) in heterophils isolated from the Fayoumi line were higher than heterophils from either Leghorn or broiler lines (Redmond et al., 2009); however, expression of IL-8, a pro-inflammatory chemokine, was higher in heterophils from Leghorns compared to those isolated the Fayoumi line (Redmond et al., 2011b). There are numerous studies reported in the literature demonstrating the role of cytokine and chemokine mRNA expression in tissues and their relationship to resistance and/or susceptibility to disease, but studies on specific tissues are outside the scope of the present review and will not be discussed herein.

4. Receptors and signaling

Defense of the host against pathogens such as *Salmonella* is initiated by the innate immune system, which in turn aids in the direction of the development of the acquired immune response. The recognition of microbial pathogens by cells of the innate immune system is accomplished by recognition of conserved molecular patterns associated with different classes of pathogens (Akira, 2001; Janeway & Medzhitov, 2002). These molecular patterns, pathogen-associated molecular patterns (PAMPs), are recognized by a family of receptors related to the IL-1 receptor (IL-1R) (TLRs; Akira, 2001; Janeway & Medzhitov, 2002; Kawai and Akira, 2010). Avian heterophils have been shown to be the primary responders to *Salmonella* infections in chickens and turkeys (Kogut et al., 1994). The importance of the avian heterophil in innate immune responses to bacterial infections has been highlighted by the observed expression of TLRs by avian heterophils (Iqbal et al., 2005; Kogut et al., 2005a,b; Philbin et al., 2005). Heterophils express TLRs 1/6/10, TLR 2 (types 1 and 2), TLR 3, TLR 4, TLR 5, and TLR 7 (Kogut et al., 2005a,b). Although TLR 9 has not been identified in the chicken, chicken heterophils and monocytes respond to the TLR 9 agonist CpG DNA in vitro (He et al., 2006; Kogut et al., 2006). Recently, TLR 21 has been described in the chicken and appears to be the functional homologue of TLR 9 in mammals, explaining the observed response to CpG by heterophils in vitro despite the lack of evidence for the existence of TLR 9 (Brownlie et al., 2009; Mackinnon et al., 2009; He et al., 2007).

TLR4 recognizes LPS, TLR5 responds to flagellin, and TLR15 is a chicken-specific TLR (Higgs et al., 2006). Evaluation of heterophils from line A and B chickens show there are no differences in mRNA expression levels of either TLR4 or 5; however, there is a significantly higher level of TLR15 in line A heterophils prior to and following stimulation with *S. enteritidis* (Nerren et al., 2009) indicating TLR15 contributes to the differential responses observed between lines A and B. The expression of TLR4 also varies among heterophils from different breeds of chickens with Leghorns having the highest expression over the Fayoumi line and broilers following *in vitro* stimulation with *S. enteritidis* (Redmond et al., 2009).

In addition to TLRs, other pathogen recognition receptors have been identified on avian heterophils. A c-type lectin receptor (CLR), Dectin-1, has been indirectly identified in the heterophil (Nerren et al., 2009). In mammals, Dectin-1 has been identified as a receptor for β -glucan (a component of fungal and bacterial cell walls). β -glucan has been shown to increase heterophil functions when given as a feed additive to poultry (Lowry et al., 2005). Further evidence for the presence of Dectin-1 on chicken heterophils was observed when the Dectin-1 specific agonist, curdlan, was applied to heterophils *in vitro* and the oxidative burst of treated cells was significantly greater than that of untreated cells (Nerren et al., 2009). In addition, laminarin, a dectin-1 inhibitor, significantly reduced the oxidative burst of heterophils treated with curdlan (Nerren et al., 2009). The findings strongly indicate the presence of a Dectin-1 or Dectin-1-like receptor on avian heterophils.

Heterophils are key cellular components involved in avian signaling cascades and host genetics can and does impact the outcome of those signaling events. Protein tyrosine kinase (PTK) activation is one of the initial steps for the induction of virtually all signaling cascades which ultimately leads to mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF- κ B) activation and subsequent production of inflammatory effector molecules (Cloutier et al., 2007; Liu et al., 2007; Vitiello et al., 2004). Studies in different broiler lines found that heterophils from line A and B chickens have notable differences in their signaling pathways following exposure to *S. enteritidis*. Line A heterophils have significantly higher levels of total phosphorylated PTK, and p38

protein production (a member of the MAPK family) while line B heterophils have higher levels of JNK protein expression (MAPK family) (Swaggerty et al., 2011b). A more detailed analysis of heterophils from lines A and B revealed differences throughout the TLR pathway including differences in key receptors (TLR4, TLR15, TLR21), adaptor molecules (TRAF3, TIRAP, IRAK4), kinases ($\text{I}\kappa\text{K}\alpha$, $\text{I}\kappa\text{K}\beta$, TAK1, p38), transcription factors (NF- κ B2, IRF7), and effector molecules (IL-6, IL-12A, CCL4, CCL5, IFN- α) indicating the heterophil response in line A birds involves coordination of genes from all components of the intracellular TLR signaling pathway (Chiang et al., 2008; Kogut et al., 2012a,b,c,d; Swaggerty et al., 2005a). Turkey heterophils also function via PTK and MAPK signaling cascades and as might be expected, differences between wild-type and commercial turkeys has been observed (Genovese et al., 2007). The wild-type turkey heterophils had significantly higher levels of total PTK, p38, and ERK (MAPK member) compared to heterophils from a commercial meat turkey.

5. Regulation of heterophil functions

The immune functions of heterophils are regulated by complex multilayers of cellular components, including receptors, intracellular signaling proteins, transcription factors, and effector molecules such as cytokines and chemokines. In addition, cellular kinases play a central role in regulating the overall activity, fine tuning the degree and specificity of immune responses. Early studies have shown that the immune functions, including chemotaxis, adherence, phagocytosis, and bacterial killing, of chicken heterophils can be enhanced through administration of *S. enteritidis*-immune lymphokines (ILK) into either 18-d-old developing embryos or day-of-hatch chicks and pouls, which in turn increased resistance to *Salmonella* (Kogut et al., 1998a,b). Although the active components of ILK have not been identified, they likely belong to the cytokine or chemokine family (Bischoff et al., 2001). Chicken IFN-gamma has been shown to enhance heterophil phagocytic capacity, prime heterophils for greater degranulation and oxidative burst activities, and increase the expression of proinflammatory and Th1 cytokines (Kogut et al., 2001a,b, 2005a,b). Chicken IL-2 also acts to prime chicken heterophils to increase the expression of IL-8 and IL-18 during phagocytosis of *Salmonella* (Kogut et al., 2003a,b). Dietary additives, probiotic bacteria (Farnell et al., 2006; Stringfellow et al., 2011) and cationic peptides isolated from Gram-positive bacterium *Brevibacillus texanus* (Kogut et al., 2007, 2010, 2012a,b,c,d), also show augmentation of the functional activities of chicken heterophils.

Pattern recognition receptors (PRR), including TLRs, scavenger receptors, dectin-1, and mannose receptors, are critically important components of heterophil activation, acting to convey extracellular stimulant signals. These receptors recognize specific ligands and incite intracellular signaling pathway cascades, either directly inducing effector functions, such as degranulation, oxidative burst, and expression of proinflammatory cytokines and chemokines or cross talking with the G protein-coupled receptor signaling cascade, a central player in phagocyte activation (Barletta et al., 2012), to modulate effector functions. TLRs are the most important family of the innate immune receptors, responsible for recognition of pathogen associated molecule patterns (PAMP). Chicken heterophils express TLRs 1LA, 1LB, 2a, 2b, 3, 4, 5, 7, 15, and 21 (Kogut et al., 2005a,b, 2012a,b,c,d; Nerren et al., 2009). Engaging these receptors by specific PAMP activates heterophils and induces degranulation, oxidative burst, and expression of immune mediators such as cytokines and chemokines (Kogut et al., 2005a,b, 2008; He et al., 2003, 2008; Farnell et al., 2003). *In vivo* and *in ovo* administration of CpG-ODN, an agonist of TLR21, primes

and enhances chicken heterophil innate immune functions and confers greater resistance to *Salmonella* infection in newly hatched chickens (He et al., 2005, 2007; Mackinnon et al., 2009). As described earlier, Dectin-1 is the primary PRR for exogenous beta-glucan, a component of fungal and bacterial cell walls. Stimulation with the Dectin-1 specific agonist, curdlan, induces an oxidative burst in heterophils from newly hatched chickens, indicating that chicken heterophils express a functional dectin-1 (Nerren and Kogut, 2009). The scavenger receptors (SR) comprise structurally and functionally divergent groups of cell surface and secreted proteins that play an important role in innate immune defenses. Stimulation with various SR ligands revealed that only ligands of SR class B induce an oxidative burst in heterophils and that heterophil degranulation is not affected by any SR ligands (He et al., 2009). The triggering receptors expressed on myeloid cells (TREM) are another type of innate immune receptors and are involved in innate inflammatory responses. Ligation of chicken TREM-A1 with TREM-A1 specific antibody induces significant increases in the phagocytosis of *Salmonella*, degranulation, and expression of the pro-inflammatory cytokine, IL-6, and the inflammatory chemokine, CXCL2, but has no effect on the production of an oxidative burst in heterophils (Kogut et al., 2012a,b,c,d).

6. Correlation with disease resistance

Chicken lines with functionally less active heterophils are more susceptible to infections than those with highly functional heterophils including *S. enteritidis* (Ferro et al., 2004) (Swaggerty et al., 2005a) *Enterococcus gallinarum* (Swaggerty et al., 2005b) *Campylobacter jejuni* (Li et al., 2008) and *Eimeria tenella* (Swaggerty et al., 2011a). Comparisons of the Fayoumi line also showed increased resistance to *E. tenella* compared to white Leghorns (Pinard-Van Der Laan et al., 1998). Collectively, all of these studies indicate differences in heterophil-mediated signaling events and subsequent gene expression are under genetic control and contribute, in part, to either a line of birds being more resistant or susceptible to food-borne and poultry pathogens. Further studies designed to define the genes and pathways responsible for determination of greater disease resistance and subsequently heterophil functions in poultry are underway and should lead to a better understanding of the role of selection and subsequent disease resistance and its relation to desired commercial production characteristics.

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References

- Akira, S., 2001. Toll-like receptors and innate immunity. *Adv Immunol* 78, 1–56.
- Barletta, K.E., Ley, K., Mehrad, B., 2012. Regulation of neutrophil function by adenosine. *Arterioscler. Thromb. Vasc. Biol.* 32, 856–864.
- Bischoff, K.M., Pishko, E.J., Genovese, K.J., Crippen, T.L., Holtzapple, C.K., Stanker, L.H., Nisbet, D.J., Kogut, M.H., 2001. Chicken mim-1 protein, P33, is a heterophil chemotactic factor present in *Salmonella enteritidis* immune lymphokine. *J. Food Prot.* 64, 1503–1509.
- Borregaard, N., Sorensen, O.E., Theilgaard-Monch, K., 2007. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol.* 28, 340–345.
- Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y., Zychlinsky, A., 2004. Neutrophil extracellular traps kill bacteria. *Science* 303, 1532–1535.
- Brinkmann, V., Zychlinsky, A., 2007. Beneficial suicide: why neutrophils die to make NETS. *Nat. Rev. Microbiol.* 5, 577–582.
- Brockus, C.W., Jackwood, M.W., Harmon, B.G., 1998. Characterization of beta-defensin prepropeptide mRNA from chicken and turkey bone marrow. *Anim. Genet.* 29, 283–289.
- Brownlie, R., Zhu, J., Allan, B., Mutwiri, G.K., Babiuk, L.A., Potter, A., Griebel, P., 2009. Chicken TLR21 acts as a functional homologue to mammalian TLR9 in the recognition of CpG oligodeoxynucleotides. *Mol. Immunol.* 46, 3163–3170.
- Brune, K., Spitznagel, J.K., 1973. Peroxidaseless chicken leukocytes: isolation and characterization of antibacterial granules. *J. Infect. Dis.* 127, 84–94.
- Chiang, H.I., Swaggerty, C.L., Kogut, M.H., Dowd, S.E., Li, X., Pevzner, I.Y., Zhou, H., 2008. Gene expression profiling in chicken heterophils with *Salmonella enteritidis* stimulation using a chicken 44 K agilent microarray. *BMC Genomics* 9, 1–11.
- Chuammitri, P., Ostojić, J., Andreasen, C.B., Redmond, S.B., Lamont, S.J., Palić, D., 2009. Chicken heterophil extracellular traps (HETs): novel defense mechanism of chicken heterophils. *Vet. Immunol. Immunopathol.* 129, 126–131.
- Chuammitri, P., Redmond, S.B., Kimura, K., Andreasen, C.B., Lamont, S.J., Palić, D., 2011. *Vet Immunol Immunopathol.* 142, 219–227.
- Cloutier, A., Ear, T., Blais-Charron, E., Dubois, C.M., 2007. Differential involvement of NF-κB and MAP kinase pathways in the generation of inflammatory cytokines by human neutrophils. *J. Leukocyte Biol.* 81, 567–577.
- Daimon, T., Caxton-Martins, A., 1977. Electron microscopic and enzyme cytochemical studies on granules of mature chicken granular leucocytes. *J. Anat.* 123, 553–562.
- Evans, E.W., Beach, G.G., Wunderlich, J., Harmon, B.G., 1994. Isolation of antimicrobial peptides from avian heterophils. *J. Leukocyte Biol.* 56, 661–665.
- Evans, E.W., Beach, F.G., Moore, K.M., Jackwood, M.W., Glisson, J.R., Harmon, B.G., 1995. Antimicrobial activity of chicken and turkey heterophil peptides CHP1, CHP2, THP1, and THP3. *Vet. Microbiol.* 47, 295–303.
- Ermert, D., Urban, C.F., Laube, B., Goosmann, C., Zychlinsky, A., Brinkmann, V., 2009. Mouse neutrophil extracellular traps in microbial infections. *J. Innate Immun.* 1, 181–193.
- Farnell, M.B., He, H., Genovese, K., Kogut, M.H., 2003. Pharmacological analysis of signal transduction pathways required for oxidative burst in chicken heterophils stimulated by a Toll-like receptor 2 agonist. *Int. Immunopharmacol.* 3, 1677–1684.
- Farnell, M.B., Donoghue, A.M., de Los Santos, F.S., Blore, P.J., Hargis, B.M., Tellez, G., Donoghue, D.J., 2006. Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. *Poult. Sci.* 85, 1900–1906.
- Ferro, P.J., Swaggerty, C.L., Kaiser, P., Pevzner, I.Y., Kogut, M.H., 2004. Heterophils isolated from chickens resistant to extraintestinal *Salmonella enteritidis* infection express higher levels of pro-inflammatory cytokine mRNA following infection than heterophils from susceptible chickens. *Epidemiol. Infect.* 132, 1029–1037.
- Flanagan, R.S., Jaumouille, V., Grinstein, S., 2012. The cell biology of phagocytosis. *Annu. Rev. Pathol.* 7, 61–98.
- Fuchs, T.A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahne, V., Weinreich, Y., Brinkmann, V., Zychlinsky, A., 2007. Novel cell death program leads to neutrophil extracellular traps. *J. Cell Biol.* 176, 231–241.
- Fujimori, K., Yamada, M., Imai, K., 1978. Distribution of neutral and acid alpha-glucosidases of granule fractions from chicken heterophil leucocytes. *Cell Mol. Biol. Incl. Cyto. Enzymol.* 23, 391–402.
- Genovese, K.J., Moyes, R.B., Genovese, L.L., Lowry, V.K., Kogut, M.H., 1998. Resistance to *Salmonella enteritidis* gran invasion in day-old turkeys and chickens by transformed t cell line produced lymphokines. *Avian Diseases* 42, 545–553.
- Genovese, L.L., Lowry, V.K., Genovese, K.J., DeLoach, J.R., Kogut, M.H., 1999. Enhancement of phagocytosis and bacterial killing by heterophils from neonatal chicks after administration of *Salmonella enteritidis*-immune lymphokines. *Vet. Microbiol.* 65, 133–143.
- Genovese, L.L., Lowry, V.K., Genovese, K.J., Kogut, M.H., 2000. Longevity of augmented phagocytic activity of heterophils in neonatal chickens following administration of *Salmonella enteritidis*-immune lymphokines to chickens. *Avian Pathology* 29, 117–122.
- Genovese, K.J., He, H., Lowry, V.K., Kogut, M.H., 2007. Comparison of MAP and tyrosine kinase signaling in heterophils from commercial and wild-type turkeys. *Dev. Comp. Immunol.* 31, 927–933.
- Genovese, K.J., He, H., Lowry, V.K., Swaggerty, C.L., Kogut, M.H., 2006. Comparison of heterophil functions of modern commercial and wild-type Rio Grande turkeys. *Avian Pathol.* 35, 217–223.
- Gerilechaogetu, H.A., Abe, A., Kondo, Y., 2009. Extracellular signal-regulated kinase (ERK) activation in chicken heterophils stimulated with phorbol 12-myristate 13-acetate (PMA), formyl-methionylleucyl-phenylalanine (fMLP) and lipopolysaccharide (LPS). *Anim. Sci.* 80, 577–584.
- Gupta, A., Hasler, P., Gebhardt, S., Holzgreve, W., Hahn, S., 2006. Occurrence of neutrophil extracellular DNA traps (NETS) in pre-eclampsia: a link with elevated levels of cell-free DNA? *Ann. NY Acad. Sci.* 1075, 118–122.
- Gupta, A., Hasler, P., Gebhardt, S., Holzgreve, W., Hahn, S., 2005. Induction of neutrophil extracellular DNA lattices by placental microparticles and IL-8 and their presence in preeclampsia. *Hum. Immunol.* 66, 1146–1154.
- Harmon, B.G., 1998. Avian heterophils in inflammation and disease resistance. *Poult. Sci.* 77, 972–977.
- Harwig, S.S., Swiderek, K.M., Kokryakov, V.N., Tan, L., Lee, T.D., Panyutich, E.A., Aleshina, G.M., Shamova, O.V., Lehrer, R.I., 1994. Gallinacins: cysteine-rich antimicrobial peptides of chicken leukocytes. *FEBS Lett.* 342, 281–285.
- Higgs, R., Cormican, P., Cahalane, S., Allan, B., Lloyd, A.T., Meade, K., James, T., Lynn, D.J., Babiuk, L.A., O'Farrelly, C., 2006. Induction of a novel chicken Toll-like receptor following *Salmonella enterica* Serovar Typhimurium infection. *Infect. Immun.* 74, 1692–1698.

- He, H., Farnell, M.B., Kogut, M.H., 2003. Inflammatory agonist stimulation and signal pathway of oxidative burst in neonatal chicken heterophils. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 135, 177–184.
- He, H., Lowry, V.K., Swaggerty, C.L., Ferro, P.J., Kogut, M.H., 2005. In vitro activation of chicken leukocytes and in vivo protection against *Salmonella enteritidis* organ invasion and peritoneal *S. enteritidis* infection-induced mortality in neonatal chickens by immunostimulatory CpG oligodeoxynucleotide. *FEMS Immunol. Med. Microbiol.* 43, 81–89.
- He, H., Genovese, K.J., Lowry, V.K., Nisbet, D.J., Kogut, M.H., 2006. Response of nitric oxide production to CpG oligodeoxynucleotides in turkey and chicken peripheral blood monocytes. *FEMS Immunol. Med. Microbiol.* 48, 99–106.
- He, H., Genovese, K.J., Swaggerty, C.L., Nisbet, D.J., Kogut, M.H., 2007. In vivo priming heterophil innate immune functions and increasing resistance to *Salmonella enteritidis* infection in neonatal chickens by immune stimulatory CpG oligodeoxynucleotides. *Vet. Immunol. Immunopathol.* 117, 275–283.
- He, H., Genovese, K.J., Swaggerty, C.L., Nisbet, D.J., Kogut, M.H., 2008. Differential induction of nitric oxide, degranulation, and oxidative burst activities in response to microbial agonist stimulations in monocytes and heterophils from young commercial turkeys. *Vet. Immunol. Immunopathol.* 123, 177–185.
- He, H., MacKinnon, K.M., Genovese, K.J., Nerren, J.R., Swaggerty, C.L., Nisbet, D.J., Kogut, M.H., 2009. Chicken scavenger receptors and their ligand-induced cellular immune responses. *Mol. Immunol.* 46, 2218–2225.
- Iqbal, M., Philbin, V.J., Smith, A.L., 2005. Expression patterns of chicken Toll-like receptor mRNA in tissues, immune cell subsets and cell lines. *Vet. Immunol. Immunopathol.* 104, 117–127.
- Janeway, Jr., C.A., Medzhitov, R., 2002. Innate immune recognition. *Annual Review of Immunology* 20, 197–216.
- Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.
- Klebanoff, S.J., 2005. Myeloperoxidase: friend and foe. *J. Leukocyte Biol.* 77, 598–625.
- Kogut, M.H., McGruder, E.D., Hargis, B.M., Corrier, D.E., DeLoach, J.R., 1994. Dynamics of the avian inflammatory response to *Salmonella*-immune lymphokines. Changes in avian blood leukocyte populations. *Inflammation* 18, 373–388.
- Kogut, M.H., McGruder, E.D., Hargis, B.M., Corrier, D.E., DeLoach, J.R., 1995a. In vivo activation of heterophil function in chickens following injection with *Salmonella enteritidis*-immune lymphokines. *J. Leukocyte Biol.* 57, 56–62.
- Kogut, M.H., McGruder, E.D., Hargis, B.M., Corrier, D.E., DeLoach, J.R., 1995b. Characterization of the pattern of inflammatory cell influx in chicks following the intraperitoneal administration of live *Salmonella enteritidis* and *Salmonella enteritidis*-immune lymphokines. *Poult. Sci.* 74, 8–17.
- Kogut, M.H., Holtzapple, C., Lowry, V.K., Genovese, K., Stanker, L.H., 1998a. Functional responses of neonatal chicken and turkey heterophils following stimulation by inflammatory agonists. *Am. J. Vet. Res.* 59, 1404–1408.
- Kogut, M.H., Lowry, V.K., Moyes, R.B., Bowden, L.L., Bowden, R., Genovese, K., Deloach, J.R., 1998b. Lymphokine-augmented activation of avian heterophils. *Poult. Sci.* 77, 964–971.
- Kogut, M.H., Pishko, E.J., Kaspers, B., Weining, K.C., 2001a. Modulation of functional activities of chicken heterophils by recombinant chicken IFN-gamma. *J. Interferon Cytokine Res.* 21, 85–92.
- Kogut, M.H., Genovese, K.J., Lowry, V.K., 2001b. Differential activation of signal transduction pathways mediating phagocytosis, oxidative burst, and degranulation by chicken heterophils in response to stimulation with opsonized *Salmonella enteritidis*. *Inflammation* 25, 7–15.
- Kogut, M.H., Rothwell, L., Kaiser, P., 2003a. Differential regulation of cytokine gene expression by avian heterophils during receptor-mediated phagocytosis of opsonized and nonopsonized *Salmonella enteritidis*. *J. Interferon Cytokine Res.* 23, 319–327.
- Kogut, M.H., Rothwell, L., Kaiser, P., 2003b. Priming by recombinant chicken interleukin-2 induces selective expression of IL-8 and IL-18 mRNA in chicken heterophils during receptor-mediated phagocytosis of opsonized and nonopsonized *Salmonella enterica* serovar enteritidis. *Mol. Immunol.* 40, 603–610.
- Kogut, M.H., Iqbal, M., He, H., Philbin, V., Kaiser, P., Smith, A., 2005a. Expression and function of Toll-like receptors in chicken heterophils. *Dev. Comp. Immunol.* 29, 791–807.
- Kogut, M.H., Rothwell, L., Kaiser, P., 2005b. IFN-gamma priming of chicken heterophils upregulates the expression of proinflammatory and Th1 cytokine mRNA following receptor-mediated phagocytosis of *Salmonella enterica* serovar enteritidis. *J. Interferon Cytokine Res.* 25, 73–81.
- Kogut, M.H., Swaggerty, C., He, H., Pevzner, I., Kaiser, P., 2006. Toll-like receptor agonists stimulate differential functional activation and cytokine and chemokine gene expression in heterophils isolated from chickens with differential innate responses. *Microbes Infect.* 8, 1866–1874.
- Kogut, M.H., Genovese, K.J., He, H., Li, M.A., Jiang, Y.W., 2007. The effects of the BT/TAMUS 2032 cationic peptides on innate immunity and susceptibility of young chickens to extraintestinal *Salmonella enterica* serovar Enteritidis infection. *Int. Immunopharmacol.* 7, 912–919.
- Kogut, M.H., Genovese, K.J., He, H., Kaiser, P., 2008. Flagellin and lipopolysaccharide up-regulation of IL-6 and CXCLi2 gene expression in chicken heterophils is mediated by ERK1/2-dependent activation of AP-1 and NF-kappaB signaling pathways. *Innate Immun.* 14, 213–222.
- Kogut, M.H., He, H., Genovese, K.J., Jiang, Y.W., 2010. Feeding the BT cationic peptides to chickens at hatch reduces cecal colonization by *Salmonella enterica* serovar Enteritidis and primes innate immune cell functional activity. *Foodborne. Pathog. Dis.* 7, 23–30.
- Kogut, M.H., Genovese, K.J., He, H., Swaggerty, C.L., Jiang, Y.W., 2012a. BT cationic peptides: small peptides that modulate innate immune responses of chicken heterophils and monocytes. *Vet. Immunol. Immunopathol.* 145, 151–158.
- Kogut, M.H., Chiang, H.I., Swaggerty, C.L., Pevzner, I.Y., Zhou, H., 2012b. Gene expression analysis of Toll-like receptor pathways in heterophils from genetic chicken lines that differ in their susceptibility to *Salmonella enteritidis*. *Front. Genet.* 3, 121.
- Kogut, M.H., Genovese, K.J., Nerren, J.R., He, H., 2012c. Effects of avian triggering receptor expressed on myeloid cells (TREM-A1) activation on heterophil functional activities. *Dev. Comp. Immunol.* 36, 157–165.
- Kogut, M.H., Chiang, H.-I., Swaggerty, C.L., Pevzner, I.Y., Zhou, H., 2012d. Gene expression analysis of Toll-like receptor pathways in heterophils from genetic chicken lines that differ in their susceptibility to *Salmonella enteritidis*. *Front. Epigenomics* 3, 1–10.
- Li, X., Swaggerty, C.L., Kogut, M.H., Chiang, H., Wang, Y., Genovese, K.J., He, H., Stern, N.J., Pevzner, I.Y., Zhou, H., 2008. The paternal effect of *Campylobacter jejuni* colonization in ceca in broilers. *Poult. Sci.* 87, 1742–1747.
- Lippolis, J.D., Reinhardt, T.A., Goff, J.P., Horst, R.L., 2006. Neutrophil extracellular trap formation by bovine neutrophils is not inhibited milk. *Vet. Immunol. Immunopathol.* 113, 248–255.
- Liu, Y., Shepherd, E.G., Nelin, L.D., 2007. MAPK phosphatases – regulating the immune response. *Nat. Rev. Immunol.* 7, 202–212.
- Lowry, V.K., Genovese, K.J., Bowden, L.L., Kogut, M.H., 1997. Ontogeny of the phagocytic and bactericidal activities of turkey heterophils and their potentiation by *Salmonella enteritidis*-immune lymphokines. *FEMS Immunol. Med. Microbiol.* 19, 95–100.
- Lowry, V.K., Farnell, M.B., Ferro, P.J., Swaggerty, C.L., Bahl, A., Kogut, M.H., 2005. Purified B-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovars Enteritidis. *Int. J. Food Microbiol.* 98, 309–318.
- Mackinnon, K.M., He, H., Swaggerty, C.L., McReynolds, J.L., Genovese, K.J., Duke, S.E., Nerren, J.R., Kogut, M.H., 2009. *In ovo* treatment with CpG oligodeoxynucleotides decreases colonization of *Salmonella enteritidis* in broiler chickens. *Vet. Immunol. Immunopathol.* 127 (3–4), 371–375.
- Macrae, E.K., Spitznagel, J.K., 1975. Ultrastructural localization of cationic proteins in cytoplasmic granules of chicken and rabbit polymorphonuclear leukocytes. *J. Cell Sci.* 17, 79–94.
- Maxwell, M.H., 1984. The distribution and localisation of acid trimetaphosphatase in developing heterophils and eosinophils in the bone marrow of the fowl and the duck. *Cell Tissue Res.* 235, 171–176.
- Maxwell, M.H., Robertson, G.W., 1998. The avian heterophil leucocyte: a review. *World's Poult. Sci. J.* 54, 155–178.
- Nerren, J.R., Swaggerty, C.L., Mackinnon, K.M., Genovese, K.J., He, H., Pevzner, I., Kogut, M.H., 2009. Differential mRNA expression of the avian-specific toll-like receptor 15 between heterophils from *Salmonella*-susceptible and -resistant chickens. *Immunogenetics* 61, 71–77.
- Nerren, J.R., Kogut, M.H., 2009. The selective Dectin-1 agonist, curdlan, induces an oxidative burst response in chicken heterophils and peripheral blood mononuclear cells. *Vet. Immunol. Immunopathol.* 127, 162–166.
- Nixon, J.B., McPhail, L.C., 1999. Protein kinase C (PKC) isoforms translocate to Triton-insoluble fractions in stimulated human neutrophils: correlation of conventional PKC with activation of NADPH oxidase. *J. Immunol.* 163, 4574–4582.
- Osculati, F., 1970. Fine structural localization of acid phosphatase and arylsulfatase in the chick heterophil leucocytes. *Z. Zellforsch. Mikrosk. Anat.* 109, 398–406.
- Philbin, V.J., Iqbal, M., Boyd, Y., Goodchild, M.J., Beal, R.K., Bumstead, N., Young, J., Smith, A.L., 2005. Identification and characterization of a functional, alternatively spliced Toll-like receptor 7 (TLR7) and genomic disruption of TLR8 in chickens. *Immunology* 114, 507–521.
- Palic, D., Ostoic, J., Andreason, C.B., Roth, J.A., 2007. Fish cast NETs: neutrophil extracellular traps are released from fish neutrophils. *Dev. Comp. Immunol.* 31, 805–816.
- Pinard-Van Der Laan, M.H., Monvoisin, J.L., Pery, P., Hamet, N., Thomas, M., 1998. Comparison of outbred lines of chickens for resistance to experimental infection with *Coccidioides* (*Eimeria tenella*). *Poult. Sci.* 77, 185–191.
- Redmond, S.B., Chuammitri, P., Andreason, C.B., Palic, D., Lamont, S.J., 2009. Chicken heterophils from commercially selected and non-selected genetic lines express cytokines differently after *in vitro* exposure to *Salmonella enteritidis*. *Vet. Immunol. Immunopathol.* 132, 129–134.
- Redmond, S.B., Chuammitri, P., Andreason, C.B., Palic, D., Lamont, S.J., 2011a. Genetic control of chicken heterophil function in advanced intercross lines: associations with novel and with known *Salmonella* resistance loci and a likely mechanism for cell death in extracellular trap production. *Immunogenetics* 63, 449–458.
- Redmond, S.B., Chuammitri, P., Andreason, C.B., Palic, D., Lamont, S.J., 2011b. Proportion of circulating chicken heterophils and CXCLi2 expression in response to *Salmonella enteritidis* are affected by genetic line and immune modulating diet. *Vet. Immunol. Immunopathol.* 140, 323–328.
- Smith, E.J., Crittenden, L.B., 1998. Genetic cellular resistance to subgroup E avian leukosis virus in slow-feathering dams reduces congenital transmission of an endogenous retrovirus encoded at locus ev 21. *Poult. Sci.* 67, 1668–1673.
- Stabler, J.G., McCormick, T.W., Powell, K.C., Kogut, M.H., 1994. Avian heterophils and monocytes: phagocytic and bactericidal activities against *Salmonella enteritidis*. *Vet. Microbiol.* 38, 293–305.

- Stringfellow, K., Caldwell, D., Lee, J., Mohnl, M., Beltran, R., Schatzmayr, G., Fitz-Coy, S., Broussard, C., Farnell, M., 2011. Evaluation of probiotic administration on the immune response of coccidiosis-vaccinated broilers. *Poult. Sci.* 90, 1652–1658.
- Swaggerty, C.L., Pevzner, I.Y., Ferro, P.J., Crippen, T.L., Kogut, M.H., 2003a. Association between in vitro heterophil function and the feathering gene in commercial broiler chickens. *Avian Pathol.* 32, 483–488.
- Swaggerty, C.L., Pevzner, I.Y., Lowry, V.K., Farnell, M.B., Kogut, M.H., 2003b. Functional comparison of heterophils isolated from commercial broiler chickens. *Avian Pathol.* 32, 95–102.
- Swaggerty, C.L., Kogut, M.H., Ferro, P.J., Rothwell, L., Pevzner, I.Y., Kaiser, P., 2004. Differential cytokine mRNA expression in heterophils isolated from *Salmonella*-resistant and -susceptible chickens. *Immunology* 113, 139–148.
- Swaggerty, C.L., Ferro, P.J., Pevzner, I.Y., Kogut, M.H., 2005a. Heterophils are associated with resistance to systemic *Salmonella enteritidis* infection in genetically distinct lines of chickens. *FEMS Immunol. Med. Microbiol.* 43, 149–154.
- Swaggerty, C.L., Lowry, V.K., Ferro, P.J., Pevzner, I.Y., Kogut, M.H., 2005b. Disparity in susceptibility to vancomycin-resistant *Enterococcus* organ invasion in commercial broiler chickens that differ in innate immune responsiveness. *Food Agric. Immunol.* 16, 1–15.
- Swaggerty, C.L., He, H., Genovese, K.J., Kaiser, P., Pevzner, I.Y., Kogut, M.H., 2006a. The feathering gene is linked to degranulation and oxidative burst not cytokine/chemokine mRNA expression levels or *Salmonella enteritidis* organ invasion in broilers. *Avian Pathol.* 35, 465–470.
- Swaggerty, C.L., Kaiser, P., Rothwell, L., Pevzner, I.Y., Kogut, M.H., 2006b. Heterophil cytokine mRNA profiles from genetically distinct lines of chickens with differential heterophil-mediated innate immune responses. *Avian Pathol.* 35, 102–108.
- Swaggerty, C.L., Genovese, K.J., He, H., Duke, S.E., Pevzner, I.Y., Kogut, M.H., 2011a. Broiler breeders with an efficient innate immune response are more resistant to *Escherichia coli*. *Poult. Sci.* 90, 1014–1019.
- Swaggerty, C.L., He, H., Genovese, K.J., Pevzner, I.Y., Kogut, M.H., 2011b. Protein tyrosine kinase and mitogen-activated protein kinase signaling pathways contribute to differences in heterophil-mediated innate immune responsiveness between two lines of broilers. *Avian Pathol.* 40, 289–297.
- van Dijk, A., Veldhuizen, E.J., Haagsman, H.P., 2008. Avian defensins. *Vet. Immunol. Immunopathol.* 124, 1–18.
- van Dijk, A., Tersteeg-Zijderveld, M.H., Tjeerdsma-van Bokhoven, J.L., Jansman, A.J., Veldhuizen, E.J., Haagsman, H.P., 2009. Chicken heterophils are recruited to the site of *Salmonella* infection and release antibacterial mature Cathelicidin-2 upon stimulation with LPS. *Mol. Immunol.* 46, 1517–1526.
- Wells, L.L., Lowry, V.K., DeLoach, J.R., Kogut, M.H., 1998. Age-dependent phagocytosis and bactericidal activities of the chicken heterophil. *Dev. Comp. Immunol.* 22, 103–109.
- Vitiello, M., D'Isanto, M., Galdiero, M., Raieta, K., Tortora, A., Rotondo, P., Peluso, L., 2004. Interleukin-8 production by THP-1 cells stimulated by *Salmonella enterica* serovar Typhimurium porins is mediated by AP-1, NF-[kappa]B and MAPK pathways. *Cytokine* 27, 15–24.
- Winterbourn, C.C., Kettle, A.J., 2012. Redox reactions and microbial killing in the neutrophil phagosome. *Antioxid. Redox Signal.* <http://dx.doi.org/10.1089/ars.2012.4827>.